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Allicin from garlic neutralizes the hemolytic activity of intra- and extra-cellular pneumolysin O *in vitro*

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ABSTRACT

Pneumolysin (PLY) is a key virulence factor contributes to the pathogenesis of *Streptococcus pneumoniae*. In this study we investigated the effect of allicin and aqueous garlic extracts on hemolytic activity of PLY both in prelysed and intact cells. Additionally the antimicrobial activity of allicin was tested against the bacteria. All tested materials potently inhibited the PLY hemolytic activity. Allicin neutralizes PLY in a concentration- and time-dependent manner. Twenty five minute incubation of PLY (2 HU/mL) with 0.61 μM/mL concentration of allicin, totally inhibited hemolytic activity of PLY (IC50 = 0.28 μM/mL). The inhibitory activity of old extract of garlic was similar to pure allicin (IC50 = 50.46 μL/mL; 0.31 μM/mL; $P < 0.05$). In contrast fresh extract of garlic inhibits the PLY hemolytic activity at lower concentrations (IC50 = 13.96 μL/mL; 0.08 μM/mL allicin). Exposure of intact cells to allicin (1.8 μM) completely inhibited hemolytic activity of PLY inside bacterial cells. The inhibitory effect of the allicin was restored by addition of reducing agent DTT at 5 mM, proposing that allicin likely inhibits the PLY by binding to cysteinyl residue in the binding site. The MIC value of allicin was determined to be 512 μg/mL (3.15 μM/mL). These results indicate that PLY is a novel target for allicin and may provide a new line of investigation on pneumococcal diseases in the future.

1. Introduction

Streptococcus pneumoniae causes several important human diseases, including serious invasive diseases like as pneumonia, septicemia and meningitis, in addition to otitis media and acute sinusitis (Musher, 1992). This organism produces several potential virulence factors responsible for this broad range of diseases (Mitchell, 2000). Pneumolysin (PLY) is a key multifunctional virulence factor contributes to the pathogenesis of pneumococcal diseases and produces by virtually all clinical isolates of *S. pneumoniae* (Cockeran et al., 2002, 2003; Feldman and Anderson, 2009).

Several lines of evidence through both *in vitro* and *in vivo* studies have revealed the potential role of PLY in the pathogenesis of pneumococcal infections.

In vitro studies demonstrated that PLY is toxic to mainly all human cell types including pulmonary alveolar epithelial cells (Rubins et al., 1993) and brain ciliated ependymal cells (Mohammed et al., 1999) as well as neuronal cells (Braun et al., 2007; Cockeran et al., 2002). It is capable of inhibiting polymorphonuclear cell respiratory burst and chemotaxis, inhibiting antibody production by human lymphocytes in sublytic concentrations. Furthermore, pneumolysin contributes to pathogenesis of pneumococcal diseases by its pro-inflammatory activities; it promotes the production and release of inflammatory factors like as IL-8, IL-6, IL-1β and TNFα (Feldman and Anderson, 2007). Pneumolysin also activates the complement system directly (Paton et al., 1984). Similarly, a direct link between severity of invasive pneumococcal diseases and pneumolysin has been shown in experimental models. It has been demonstrated that PLY-deficient *S. pneumoniae* injected in mice was less virulent

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